IMMUNOLOGIC PARAMETERS OF HIV

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The immunology laboratory can be used to sort out questions of HIV disease pathogenesis. We are still not entirely clear as to the mechanisms of immune deficiency in persons with HIV infection. But having the opportunity to intervene with potent antiretroviral therapies gives us an opportunity to change the dynamic equilibrium, and by inducing this change and monitoring the mechanisms of immunologic restoration, we can gain some understanding of the actual mechanisms of immune deficiency.

One of the things we can do in terms of monitoring is to define both the magnitude and the nature of immune responses during antiviral therapies. Using the laboratory can give us the opportunity to identify good predictors of serologic, clinical, and immunologic response, and this feeds back into the opportunity to define the pathogenesis of the disease. The mechanisms of immune restoration may help us define the mechanisms of immune deficiency in people with HIV disease. We have been able to characterize immune deficiency in HIV infection for some time. We know what it looks like, but we do not know entirely how it comes to pass. There are both qualitative and quantitative impairments in immune function in persons with HIV disease. With these qualitative and quantitative defects, we have the attendant risk of opportunistic infections that define AIDS. A thesis again is that immune activation plays an important role in the pathogenesis of immune deficiency in AIDS. It seems somewhat counterintuitive that persons with immune deficiency have evidence of immune activation, but certainly phenotypically and functionally that is what it looks like.

How would immune activation play a role in disease pathogenesis? There are several ways to look at this. One is that T cell activation is required for a complete HIV replication cycle. A resting CD4+ cell is not susceptible to HIV infection and there are numerous sites in the viral life cycle where infection is aborted in a cell that is not activated. An activated cell is required to permit and potentiate viral replication. Additionally, there is evidence of immune activation such as heightened production of proinflammatory cytokines. Plasma levels of the proinflammatory cytokines tumor necrosis factor alpha (TNF) and interleukin 6 are substantially elevated and with infection, continue to elevate as disease progresses, and spike in the setting of opportunistic infections. Even among persons with stable HIV infection, plasma levels of both TNF and interleukin 6 correlate well with plasma levels of HIV 1 RNA.

An intriguing observation made by a number of investigators but explored more fully by Janice Giorgi is the high level activation antigen expression of CD38 on CD8+ T cells. This may give us a clue as to the mechanism by which HIV replication induces immune deficiency. There is a reasonable correlation between CD38 density on CD8+ T cells and plasma levels of HIV 1 RNA. This suggests that these two indices are associated in some way. More importantly, in a multivariate analysis, Dr. Giorgi was able to show that not only did CD38 density predict clinical outcome and correlate with HIV RNA, but it was an independent predictor that correlated better with disease outcome than did plasma levels of HIV 1 RNA.

This suggests that viral replication induces immune activation which itself induces cell loss, maybe through programmed cell death or through additional mechanisms. This observation is certainly consistent with that speculation.

Treatment trials in the AIDS clinical trials group have been used to sort out some of these potential mechanisms. One study was an AIDS clinical trials group protocol 315 which enrolled 53 patients with CD4+ cell counts between 100-300, indicating these persons had moderately advanced disease. All had received AZT in the past but none had received 3TC or Ritonavir and they had a five-week antiviral washout. On day zero, they were started on antiretroviral therapies with the protease inhibitor and on day ten AZT and 3TC were added. They had an expected biphasic response in their viral RNA levels with a median drop of about 2.8 logs from baseline. The lower limit of detection was 100 copies/mL, and half of the patients had viral RNAs below the limit of detection. What happens immunologically in these moderately advanced patients is well known. There is a biphasic return of circulating lymphocytes as measured in total lymphocyte counts and in CD3+ T-cells. Increases in the CD19+ B cell counts are similar whereas the number of cells with a phenotype of natural killer cells was entirely stable, i.e., unchanged by the application of antiviral therapies. CD4+ lymphocyte subsets can be defined by the expression of surface markers identified as naive CD45 RA positive 62L positive populations or CD45Rt negative CD45Rt positive memory populations. We see a biphasic increase in both populations with the rapid first phase increase within the first 4-8 weeks, and a slower second phase increase in total CD4+ cells of both memory and naive phenotypes.

The story is a little different when you look at CD8+ cells. There is a first phase rapid increase, and then a second phase decrease in the total numbers of circulating CD8+ cells. Examining the subsets of these lymphoid populations, there is a first phase increase in the memory populations and then over the second phase of therapy from about week 8 to 48, there is a progressive loss in the total numbers of circulating memory CD8+ cells. The total numbers of naive cells tend to rise over this period of time. What is responsible for these first and second phase changes? Are these increases a consequence of thymic maturation? Why do we lose cells?

At this point, we have some information that may be useful in this regard with respect to the ability to examine the average telomere lengths of patient cells as a marker of their replicative history. Average telomere lengths are evaluated by taking DNA from cells, cutting it up with a restriction enzyme, and measuring the average length of the telomeres with probes that correspond to their exomeric repeats. What was seen in this population was surprising: In eleven patients enrolled in ACTG315 who were on antiretroviral therapies for a period of 48 weeks, the changes in the average telomere lengths of the CD4+ cell populations were diverse. As a group, there was no change in the lengths of the CD4+ cell telomeres. On the other hand, in the CD8+ cells, average telomere lengths actually increased significantly. What this probably represents is a combination of the loss of senescing cells, and their replacement by cells that are phenotypically naive and are junior in terms of their replicative experience. Studies from several groups now have shown that a large proportion of those expanded CD8 cells are HIV reactive, and those are the cells that tend to decrease in frequency. But, we do not observe a first phase rise in circulating CD8+ cells in all populations of patients receiving antiviral therapies. For example, there are some preliminary data from ICC004 which is a study of AZT, 3TC and Indinavir among patients with CD4+ cell counts >500 and low viral RNAs (<10,000 copies/mL). The study reported by Alan Landay shows a progressive loss of CD8+ cells rather than the first phase rise we saw in ACTG315. Is it because patients received a different protease inhibitor, or is it related to the stage of the disease? The answer is probably the latter, as seen in data extracted from ACTG320 which is a study that was conducted among patients in an advanced state of disease similar to the ACTG315 population. These patients had CD4+ cell counts <200 and whether they received AZT and 3TC or AZT and 3TC plus Indinavir, there was a first phase rise in the total numbers of circulating CD8+ cells. This finding was more pronounced in the population receiving AZT and 3TC plus Indinavir.

What does the first phase rise represent? We do not see it in patients in early disease. We see it in patients with advanced disease and we seem to see it irrespective of the specifics of antiviral therapy. We suspect this represents some degree of cellular redistribution and we believe the site of activity is in the lymph nodes. One reason to postulate this is that the only cell population in which there is no first phase rise was among natural killer cells. Of all the cells that we have been looking at B cells, T cells, naive and memory cells, natural killer cells are the ones that do not spend much time in lymphoid tissues, and they were virtually unaffected during the first phase rise of other cell populations.

What happens functionally with treatments for HIV disease? Fortunately, things look better functionally as well when we look at delayed type hypersensitivity (DTH) in patients receiving highly active antiretroviral therapies. Over the 48 weeks of therapy, there was a progressive increase in the DTH responses from 9% responses to a single antigen at baseline to the point where approximately 16% of patients had two responses and 20% had one DTH response at the end of 48 weeks of therapy. These are significant changes. There was essentially no change in lymphocyte proliferation over 48 weeks of therapy in response to tetanus toxoid. These patients were not immunized over this period, but they were immunized at 48 weeks and in the future we hope to report responses after immunization.

Similar responses were seen in terms of LPA responses to streptokinase. However, with Candida, we observed an enhancement of the LPA responses over the 48 weeks of therapy. By the end of therapy, the responses of our patients were not different from the responses among healthy, HIV sero-negative controls. Likewise, Brigitte Autran has shown dramatic enhancement of LPA responses to CMV antigens and antigens derived from Mycobacterium tuberculosis (MTB) in persons receiving HAART regimens. These antigens, MTB and CMV, were likely present in the study population due to immunization in the former and infection in the latter. These observations, i.e., failure of enhancement in responses to tetanus and streptokinase and the ability to enhance responses to antigens that are invariably present, suggest some important possibilities. First, the concept that antigen driven activation is responsible for cell loss in HIV disease is probably wrong. Antigen driven T cell activation resulting in cell loss of specific clones that are activated is probably not a tenable hypothesis. Second, the presence of antigen is probably required in order to permit some degree of enhancement of responses. I believe these observations will turn out to be extremely important.

There were essentially no enhancements in LPA responses on any one of three different HIV antigens. Our controls, of course, did not have any responses to HIV antigens, either an envelope preparation, core protein or p66. There was no change in these responses over time. However, these antigens are still present when we look at lymphoid tissue of these patients. Yet despite the presence of these antigens, there was no enhancement in LPA responses to HIV antigens. This suggests something very different is going on in terms of the pathogenesis of immune responses to these antigens. Bruce Walker and Franco Lori have generated a very nice series of observations suggesting the following. Their data are taken from a Berlin cohort of patients who had acute HIV sero-conversion illness and who received antiretroviral therapy within six months of the acquisition of HIV infection. Ordinarily, among patients who are not treated after seroconversion illness, there is no LPA response to HIV antigens. Further, in patients with chronic HIV infection, there are also no LPA responses to HIV antigens. However, in patients who are treated early there are very nice LPA responses to HIV antigens (excepting a few long term non-progressors). This suggests that early after acquisition of HIV infection is a time at which CD4+ cell dependent responses to HIV antigens can develop if HIV replication is blocked.

What happens to the T cell repertoire? If you are generating new T cells and they undergo thymic education, one would hope there is a diversification and the ultimate development of a repertoire that is very broad, potentially capable of reacting with a whole host of peptide sequences that can be bound by most MHC antigens. In both the CD4+ and the CD8+ populations in healthy controls there is a fairly broad distribution of cells of different TCR families. In contrast, often patients show dramatic expansions of some families of both CD4+ and CD8+ populations. What this represents in the CD4+ population is a very interesting question. These are patients who do not have demonstrable reactivity with any HIV antigens. What does this expansion truly represent? We do not believe they are HIV reactive, but we do not know this yet, and this is a real important question. If these cells are HIV reactive, we must explain their anergy in LPA reactions. Are they expansions in response to opportunistic pathogens? The point is that over 12 weeks (and now we have data over 48 weeks of therapy) there is not much of a change, and you do not see a normalization of the repertoire, as defined by a 3 dimensional structure in these patients. Whatever these perturbations represent, they tend to persist despite successful antiviral therapies.

Returning to the issue of immune activation, we have data on a number of indices of immune activation over time. Over 48 weeks of therapy, TNF-alpha levels in patients participating in ACTG315 fell from a median of 50 picograms/mL to 22 picograms/mL at week 48. Despite the dramatic decreases in circulating TNF, these are still not normal levels. In healthy controls levels <20 picograms/mL are the rule. Likewise, decreases in the expression of both HLA-DR and CD38 on both CD4+ and CD8+ cells were observed. These decreases were dramatic. What we learn from this is that immune activation is certainly a consequence of viral replication. These indices of immune activation fall dramatically, so it is not TNF expression at baseline that drives HIV replication but rather HIV replication is what is driving TNF and likewise, in all probability, driving immune activation in both CD4+ and CD8+ cells. What are the consequences of this?

We believe most of the activity is taking place in the lymph nodes. In studies reported by Jan Anderson, the frequency of gamma interferon positive cells decreased in tonsillar tissues after application of HAART. There was a concomitant decrease in the frequency of cells that were IL2 staining, and a tendency to observe decreases in the frequency of cells that were positive for IL4, IL10, interleukin-1 calprotectin, and CD68. In addition Pat Bucy has found that the heightened expression of adhesion molecules ICAM-18 VCAM in lymphoid tissues of HIV infected persons decrease dramatically after application of HAART. Similarly tonsillar biopsies taken from untreated HIV infected persons reveal a high frequency of apoptotic cells. In ACTG 315, within days after application of HAART, the frequency of apoptotic cells in these biopsies tended to normalize.

In summary, there is a biphasic cellular restoration that may vary with the stage of disease but probably not with specific therapy. Persons with advanced disease with high levels of immune activation and a high frequency of apoptosis are those who show a first phase response which is characterized by an increase in all lymphoid populations except natural killer cells. This increase is likely to reflect a redistribution of cells from lymphoid tissue of cells that have become less adherent and also less susceptible to undergo programmed cell death. The second phase occurs between weeks 8-48, and is characterized by increases in naive CD4+ and CD8+ cells, and decreases in memory CD8+ cells. In terms of function, there are increased helper responses to selected antigens, such as CMV, Mycobacterium tuberculosis and Candida. These are antigens from microorganisms that are likely to be present in the patient at the time of study. There is no increase in T helper responses to HIV antigens.

Some of the remaining questions are whether or not the naive cell increases are truly a result of thymic maturation. Getting to this may be facilitated both by the ability to do multi-color flow analysis to define the diversity of the repertoire among cells that are phenotypically identified as naive, and/or the ability to look at the frequency of cells in these population that contain closed circle DNA markers of recent thymic emigrants. In addition, it is very important to know whether or not lost immune responses to HIV can be restored by immunization, and if so, what are the predictors of enhanced responses? If the best predictor is the naive cell increase, then this suggests that HIV reactive cells have been lost. If on the other hand the best predictor is the memory cell increase, it would suggest that the cells have not been lost but had somehow been rendered anergic or were below a frequency that rendered them detectable. It will be important to know whether continued HIV suppression will result in continued immune restoration. That is, is there a ceiling on the magnitude of immune restoration that is possible after application of highly active antiretroviral therapy? We are now in the third year of following patients in ACTG315 to address this question.

Finally, what are the immunologic consequences of viral rebound after highly active antiretroviral therapies? Not all people who have rebound experience an immunologic deterioration. Therefore, there appears to be some benefit to HAART even in the presence of virologic failure.